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(58) Field of search
UK CL (Edition J) **B1H**
INT CL⁴ **B01D**

(54) Column for chromatography

(57) A chromatography column is formed of a number of hollow segments (12) which are positioned one on top of the other through their flat-ground faces and are pressed together by a cap nut (4) disposed on the upper part of a steel housing (1). A base segment (9) is supported on a base by feet 10 and is provided with a solvent-permeable glass frit (11).

The substance to be investigated is placed above the glass frit (11) in the base segment (9) and carried upwards into the stationary phase surrounded by the hollow segments (12) by the solvent entering between the feet (10). When the chromatographic separation is complete, the column formed by the hollow segments (12) is disassembled, and the substance components present in the individual hollow segments can be analyzed further.

FIG.1

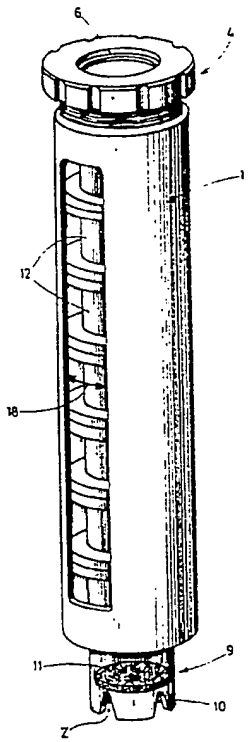

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FIG.1

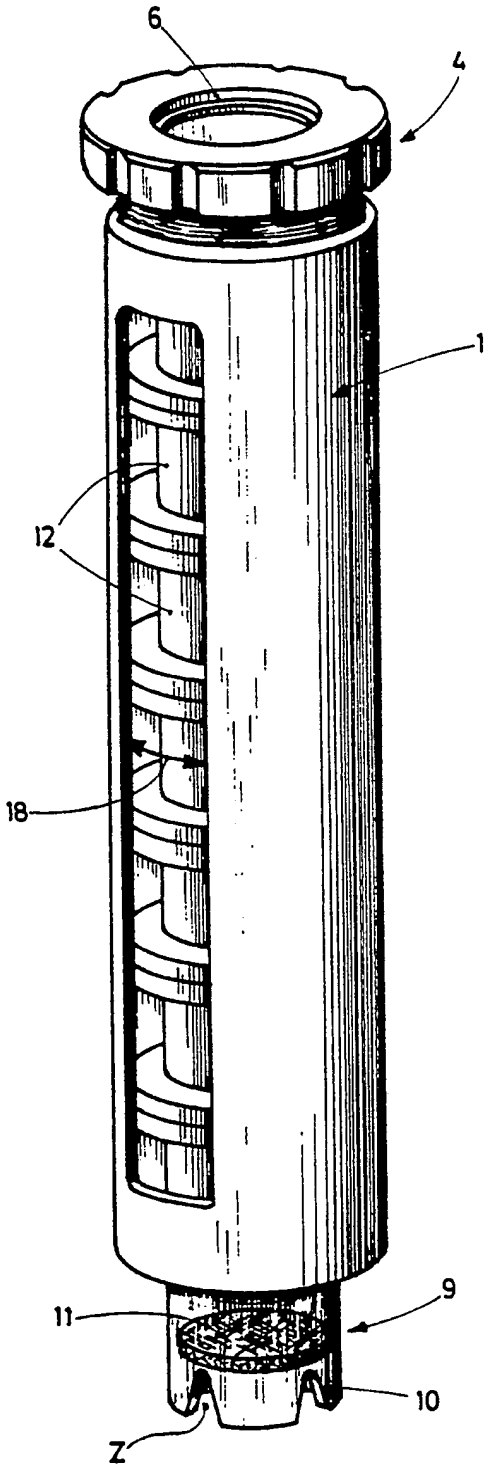

FIG.2

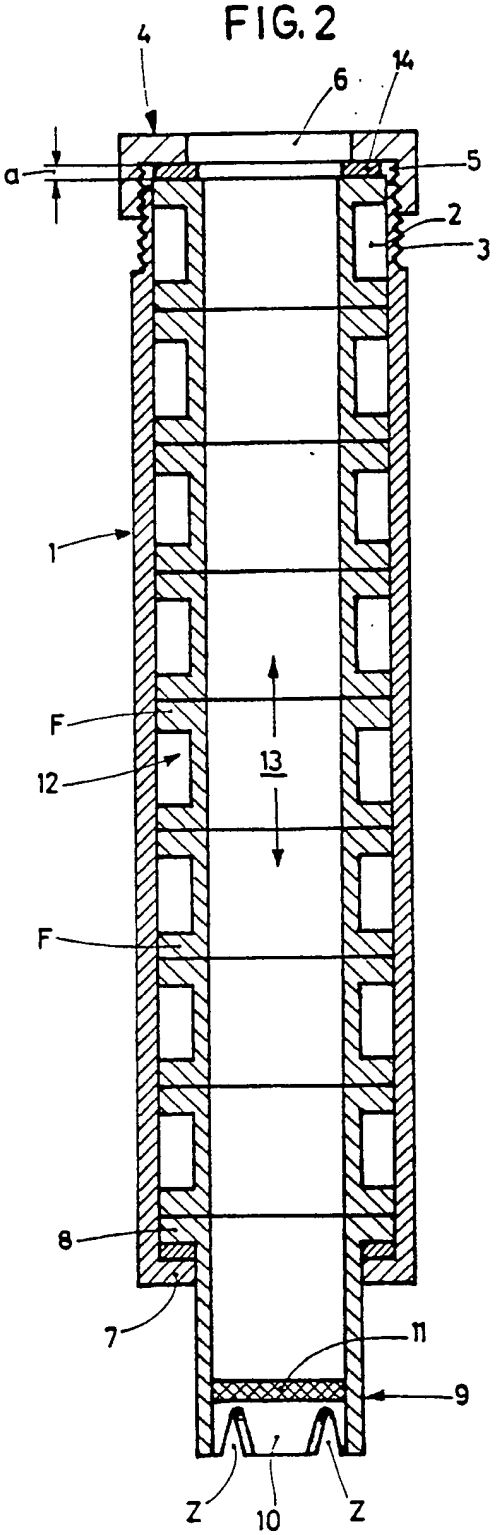

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FIG. 3

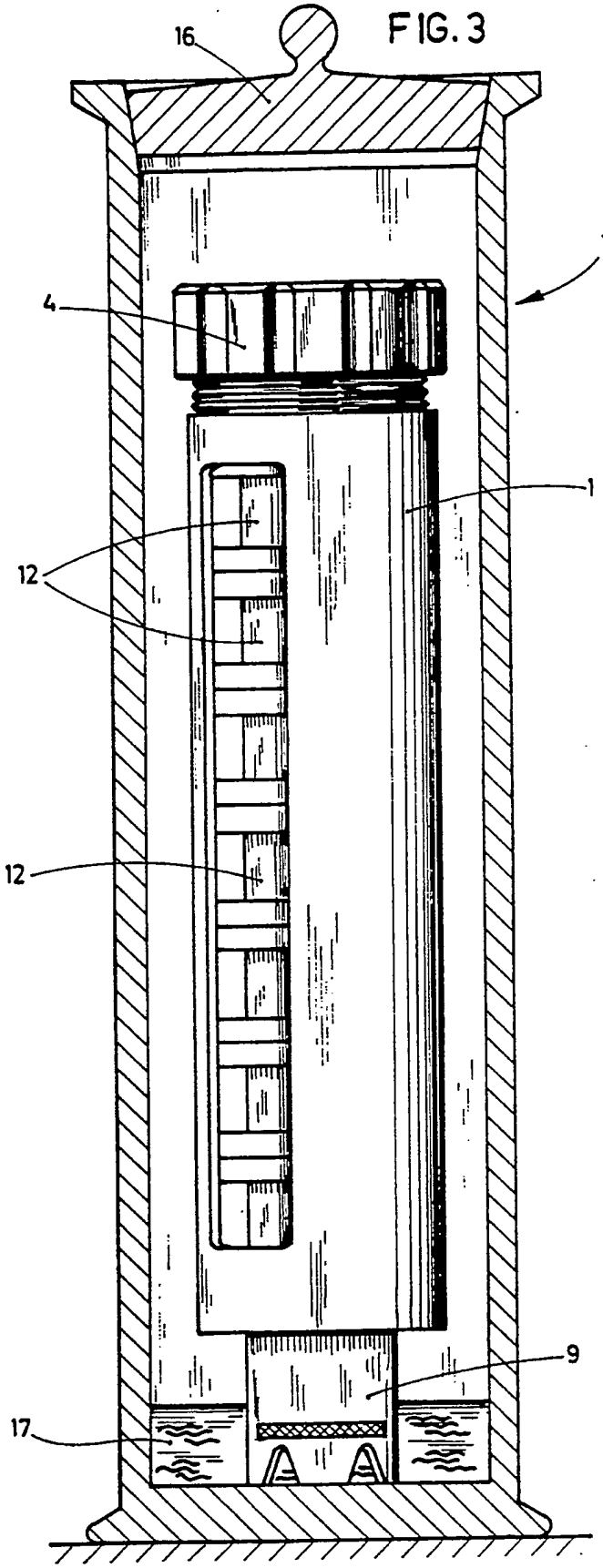
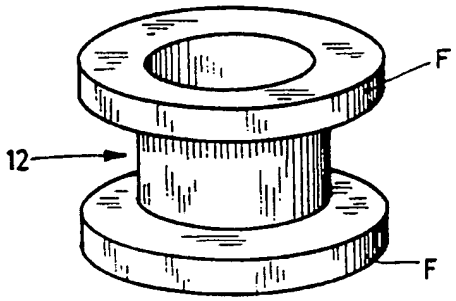


FIG. 4



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Column chromatograph, and a process for operation thereof

The present invention relates to a column chromatograph as defined in the preamble of the independent Patent Claim 1. It furthermore relates to a process for operation thereof.

The column chromatographs known at present and used for analytical and preparative capillary chromatography are, as those skilled in the art know, afflicted with numerous disadvantages, some of which are serious and the most important of which can be summarized briefly as follows.

As far as economic efficiency is concerned, it may be stated that the solvent consumption is relatively high and monitoring of the separation process by skilled personnel is rather expensive. In addition, the separation methods cannot be used directly by TLC or adapted for HPLC and MPLC. The apparatuses used are expensive, and the process is not effectively reproducible in most cases. In addition, the recovery rate is usually considerably less than 100% of all components.

The selectivity of the known column chromatographs also leaves something to be desired. Thus, it is not possible to produce specific column packings for all types of samples, from non-polar RP phases through silica gel to polar normal phases, ion exchangers based on silica gel, wide-pore and chiral phases.

A further criticism, regarding safety, is that the known columns tend to run dry or overflow since operation cannot be carried out in a closed system using a saturated chamber. In addition, the known chromatography columns cannot be left to run overnight since the capillary action is not retained in saturated chambers. Furthermore, the danger of explosion and fire due to readily volatile solvents must not be underestimated, and toxic emissions are in many cases also released to the environment.

The lack of versatility is a further disadvantage which is criticized time and time again in practice.

The object of the present invention is to eliminate these disadvantages and accordingly to propose a column chromatograph which has high flexibility in practical use compared with conventional systems and, as far as the various abovementioned aspects are concerned, provides a considerable advance with respect to saving in time and costs.

The column chromatograph according to the invention is defined in the characterizing part of the independent Patent Claim 1; the definition of the process used for operation thereof is given in Claim 9.

An illustrative embodiment of this chromatograph is described below with reference to the attached drawing, in which:

Figure 1 shows a perspective view of an embodiment of the column chromatograph,

Figure 2 shows a vertical section thereof,

Figure 3 shows a representation of the entire instrument, including the glass housing enclosing the actual column chromatograph, and

Figure 4 shows a perspective view of an individual hollow segment.

Figures 1 and 2 show a cylindrical housing 1 which is made of stainless steel and is open at its upper end and in which the section surrounding the opening 2 has a male thread 3; a cap nut 4 provided with female thread 5 and a central opening 6 can be screwed onto the male thread 3.

At its lower end, the housing 1 has an inward-facing ring flange 7 which supports the supporting flange 8, facing radially outwards, of a base segment, which, as a whole, is labelled 9. The latter is a cylindrical container which is made of borosilicate or quartz glass, is supported on a base via feet 10, and carries a disk-shaped glass frit 11 in the interior, directly adjacent to the upper edges of the feet. This glass frit 11 must be impermeable to the stationary phase used (for example pulverulent silica gel). In contrast, it must allow the

mobile phase (solvent) to permeate from outside into the interior of the base segment 9. Glass frit 11 is firmly anchored in the base segment by welding, clamping or in another manner.

Above the base segment 9, a plurality of hollow segments 12 are disposed one above the other in a manner such that their bores, positioned coaxially one above the other, form a cavity 13 which serves for accommodation of the stationary phase. Each hollow segment 12 has a cylindrical body whose two end faces are provided with ring flanges F extending radially outwards (Fig. 4). The hollow segments 12, which likewise comprise of borosilicate or quartz glass, are flat-ground on their outer end faces, so that the joints produced when the segments are placed one on top of the other are sealed so as to be liquid-tight.

The overall height of the housing 1 and that of the column formed from the individual hollow segments 12 are matched to one another in a manner such that the uppermost hollow segment extends beyond the upper edge of the housing by a distance "a". If, after insertion of a gasket 14, the cap nut 4 is screwed onto the housing 1, it presses the hollow-segment column together and thereby ensures that the column is sealed liquid-tight as desired.

As shown by Fig. 3, the housing 1 containing the hollow-segment column is preferably disposed within a glass housing 15 which can be sealed using a ground, gas-tight lid 16 and whose lower part serves as a solvent reservoir 17.

This apparatus of uncomplicated construction is employed as follows when performing capillary chromatography:

The substance to be investigated, the components of which are to be investigated quantitatively analytically and/or preparatively, is firstly mixed with a known filler, and the mixture is dried if necessary. The dry substance/filler mixture is then introduced into the base segment 9. The base segment 9 is then suspended in the housing 1 by means of its support flange 8, and the hollow segments

12 are then built up above the base segment 9 and the cavity 13 thereby formed is filled with the stationary phase (for example silica gel). After the gasket ring 14 has been placed on top of the uppermost hollow segment 12, the cap nut 4 is screwed on. The chromatography column is thus ready for operation.

The mobile phase (solvent) can be added, for example, by placing the housing 1 by means of the base segment 9 projecting below into a solvent-filled tank. In the preferred embodiment shown in Figure 3, the entire housing 1 is accommodated in the glass housing 15 in a manner such that the solvent present in the lower part of the latter can pass through the interspaces Z between the feet 10 to the frit 11, and, permeating the latter by capillary action, passes on into the interior of the base segment.

The solvent moving upwards in the stationary phase carries the components present in the substance to be investigated, which, as known to those skilled in the art, are deposited at different column levels, that is to say within different hollow segments 12, due to their different rates of travel.

When separation of the substance into its components is complete, the hollow-segment column is disassembled into the individual segments 12, which can be separated from the adjacent segments easily and cleanly thanks to their configuration.

Amongst the advantages of the process described is that the individual components are available separately when chromatographic separation is complete. The substance components which are close together in adjacent hollow segments 12 can be collected along with the pertinent portions of the stationary phase and prepared for re-separation using fresh solvent mixtures.

When the separation is complete, the substance present in the base segment 9 can also be investigated for components which are not capillary-active.

As practical experiments have already shown, all the abovementioned disadvantages of conventional processes

can be eliminated using the chromatographic method described. The advantages thus achieved can be summarized briefly as follows:

Economic efficiency:

- Less than 95% of the previous solvent consumption.
- No monitoring.
- Separation methods can be used directly by TLC or adapted for HPLC and MPLC.
- Simple equipment, easy use, but nevertheless effectively reproducible, in contrast to apparatuses which are often expensive.
- Recovery rate of virtually 100% for all components.
- Multiple reusability of the segments.

Selectivity:

- Specific column packings for all types of samples, from non-polar RP phases through silica gel to polar normal phases, ion exchangers based on silica gel, wide-pore and chiral phases.

Safety:

- The column does not run dry or overflow since it can be operated in a closed system using saturated chambers.
- Can be left to run overnight since the capillary action at the end of the column (as in TLC) is increased in saturated chambers.
- No danger of explosion and fire due to readily volatile solvents; this also means no emission into the environment.
- No pressurized operation.

Versatile:

- The appropriate column is available for sample amounts from a few milligrams up to about 2.5 grams (varies depending on the separation problem). Larger columns can be supplied on request for more capacity and larger sample volumes.

High capacity:

- Separation of preparative sample amounts by

J.T. Baker BAKERBOND 40 µm standardized, neutral washed silica gel and BAKERBOND specific, precisely defined bound phases.

High resolution:

- The uniformly compact concentration of BAKERBOND bound phases and the pure grain fractions guarantee optimum resolution, even of complex substance mixtures.

Reproducible:

- Always identical conditions due to the saturated chamber.
- Strict production and quality control guarantees constant high quality of BAKERBOND adsorbents.

A further advantage of the apparatus described is that it is also possible to use the stationary phase with grain sizes of below 40 µm without using pressure. It has been shown experimentally that extreme grain sizes of, for example, 5 µm can be employed without difficulties. The use of pressure-reinforced equipment is therefore superfluous.

According to a preferred process, the substance to be separated is firstly dissolved in a suitable solvent and subsequently mixed with the stationary phase selected. This mixture is then dried and introduced in dry form into the base segment 9.

The practical use of the instrument described is illustrated below with reference to two use examples based on the use of the hollow-segment column having 16 hollow segments.

Example 1

Test dye mixture, Merck number type 9354

1 ml of Merck dye is mixed with
10 ml of methylene chloride (dichloromethane) and
10 g of Baker silica gel (40 µm), No. 7024, the mixture is shaken thoroughly and evaporated to dryness on a rotary evaporator. The dry contents of the flask are introduced into the base segment 9 as prescribed, the latter is placed in the housing 1, and the chromatography column

desired is constructed using empty hollow segments 12 by applying pressure. The chromatography column is filled with the same quality adsorbent as indicated above and consolidated by tapping. The column is ready for separation. Development is carried out in the glass chamber using methylene chloride. When all the hollow segments 12 are saturated, the apparatus is released from the tensioning, and the hollow segments 12 are pushed up individually and are scraped flat. Each hollow segment 12 is placed in a separate glass beaker and extracted in methylene chloride by swirling, and the supernatant solutions are subjected to TLC analysis. Identical qualities are combined and filtered under suction, rinsed and concentrated or evaporated to dryness.

The sample accommodation segment permits non-destructive testing of sample substances which remain stationary under all circumstances.

The amounts of solvent saved are very considerable.

Example 2

Separation of a serum solution containing cholesterol esters, triglycerides and cholesterol.

Extractant; methylene chloride and toluene in the ratio 5:2, i.e. 75 ml of methylene chloride with 30 ml of toluene.

The column is prepared in accordance with the procedure given above (sample example No. 1).

Separation in the column using the methylene chloride toluene ratio above. Work-up in accordance with procedure (sample example No. 1). TLC monitoring by means of methylene chloride/toluene in the abovementioned ratio. Development of the TLC plates for visualization: molybdatophosphoric acid in ethanol as the spray reagent.

The particular advantage of this chromatography system is that the entire segments chromatography (dry-segment chromatography) can be carried out in an inert gas atmosphere by means of a glass chamber. As a further benefit, it is apparent that, in this process, even low-boiling

solvent mixtures do not evaporate or hardly change in ratio and/or represent an environmental pollutant.

Very considerable amounts of solvent are saved in all experiments using this capillary chromatography. Conditioning of the column becomes entirely superfluous. By pressing the segments together on screwing the steel housing together, lateral leakage is prevented. The column itself is open at the top (opening 6) in order to permit exit of air or gas as a consequence of capillary absorption of liquid, the volume not changing even in a closed chamber, as described in the previous example.

The illustrative embodiment described with reference to the enclosed drawing can be substantially modified by those skilled in the art in the context of the inventive step. Thus, it would be possible to perform chromatographic separation processes using shorter columns, for example, by equipping the housing 1 with only a few hollow segments 12, while the remainder of the housing space up to the cap nut 4 would be filled with fillers. In this way, the same housing 1 can be used for columns of different heights.

According to the required total size it will also be possible to combine several columns - of identical or differing lengths - by means of adequate coupling elements. In this way for instance, two columns having 4 and 8 segments may be combined to constitute a column with 12 segments.

The coupling element is preferably a coupling sleeve provided with an internal thread, which would have to be screwed upon the threaded extremities of the columns.

The segment shown in Fig. 4 may also be made of solid glass, whereby the annular groove shown between the two flanges F would be filled by a solid glass wall.

Patent Claims

1. Column chromatograph for analytical and/or preparative capillary chromatography, having a column which is intended for accommodation of the stationary phase and is made of a preferably at least partially transparent material, wherein the column comprises several hollow segments (12) which are located one on top of the other and sealed by means of their annular end faces and enclose a cavity (13) which accommodates the stationary phase, and wherein the lowermost hollow segment is supported on a base segment (9) which has an accommodation chamber, for the substance to be investigated, which is sealed externally at at least one point by a wall part (11) which is permeable for the mobile phase, but impermeable for the stationary phase, the column formed by all the hollow segments (12) being held together by a clamp device (1/4) in order to seal the joints between two adjacent hollow segments.

2. A column chromatograph as claimed in claim 1, wherein each of the hollow segments (12) has a cylindrical body whose two end faces are provided with ring flanges (F) extending radially outwards.

3. A column chromatograph as claimed in Claim 2, wherein the hollow segments (12) are made of borosilicate glass or quartz glass and their end faces, positioned one on top of the other, are flat-ground or provided with an O-ring made of teflon in order to provide the sealing necessary.

4. A column chromatograph as claimed in any one of Claims 1 to 3, wherein the base segment (9) likewise has a cylindrical accommodation chamber for the lowermost part of the columnar stationary phase, which chamber is terminated in a downward direction by a disk-shaped frit (11), preferably a glass frit, the mobile phase to be added outside the base segment (9) having access to the interior of the base segment (9) through the interspaces (Z) of at least two supporting feet (10) separated from one another and through

the frit (11) mentioned.

5. A column chromatograph as claimed in any one of claims 1 to 4, wherein the clamp device is a housing (1) which is made of a solvent-resistent material, encloses all the hollow segments (12) and has a cap nut (4) at its upper, open end and a ring flange (7) facing radially inwards at its lower end for accommodation of a suspended flange (8) disposed at the upper edge of the base segment (9).

6. Column chromatograph as claimed in Claim 5, wherein the housing (1) is a cylindrical steel housing provided, over the majority of its overall height, with two diametrically opposite slots (18) for removing the hollow segments (12) or for function monitoring.

7. Column chromatograph as claimed in Claim 5, wherein, in addition to the hollow segments (12) intended for accommodation of the stationary phase, the column contains a number of filler pieces so that the same housing can be used for a hollow-segment column of any desired height by bridging the space remaining between the uppermost hollow segment and the cap nut (4) by filler pieces.

8. Column chromatograph as claimed in either of Claims 5 and 6, wherein the housing (1) containing the hollow-segment column is disposed within a second housing (15) which can be sealed to be gas-tight and whose lower section (17) serves as a reservoir for the mobile phase in a manner such that the chromatograph can also operate in an inert gas medium and/or the undesired volatilization of readily volatile mobile phases is prevented.

9. A process for operating a column chromatograph as claimed in one or more of Claims 1 to 7, which comprises

a) mixing the substance to be separated with a filler and drying the mixture if necessary,

b) introducing the dry substance/filler mixture into the base segment,

c) constructing the hollow-segment column above the base segment and filling it with the stationary phase,

d) using the clamp device, including the base segment,

to press the hollow-segment column together so that it is sealed,

e) dipping the hollow-segment column, along with its lower section, the base segment projecting from the former, into the mobile phase so that the components of the substance to be separated are distributed over the individual segments of the hollow-segment column by the mobile phase arising from the base segment due to capillary action, and then

f) disassembling the hollow-segment column and evaluating the individual hollow segments, including the base segment, along with their content of stationary phase containing the particular substance component.

10. The process as claimed in Claim 9, in which, before performing the column chromatography, a preliminary experiment is carried out, for example using the thin-layer method, wherein the solution used in the preliminary experiment is also used to perform the segment-column capillary chromatography.

11. The process as claimed in Claim 9, wherein, when the chromatographic separation is complete, any substance components which are close together in adjacent hollow segments are collected, along with the pertinent portions of the stationary phase, and prepared for re-separation using fresh solvent mixtures.

12. The process as claimed in Claim 9, wherein, when the chromatographic separation is complete, the substance present in the base segment is investigated for components which are not capillary-active.

13. The process as claimed in any one of Claims 9 to 12, wherein the substance to be separated is initially dissolved in a suitable solvent, subsequently mixed with the stationary phase selected and finally dried and introduced into the base segment.

14. A column chromatograph substantially as hereinbefore described with reference to the accomapnying drawings.

15. A process according to claim 9 substantially as hereinbefore described.

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